Addendum #7 to Data Evaluation Record (DER) for MRID 42836103
Algal Toxicity, using *Skeletonema costatum*, Tier I (Guideline 122-2)

Citation: Hughes, J.S. and P.H. Balcom. 1993. The Toxicity of DCPA Technical to *Skeletonema costatum*. Laboratory Project ID No. B038-033-3. Conducted by Malcolm Pirnie, Inc., Tarrytown, NY. Submitted by ISK Biotech Corporation, Mentor, OH. EPA MRID No. 428361-03.

Guideline: 122-2 (Algal Toxicity, Tier I)

Chemical: Chlorthal Dimethyl (DCPA) (PC 078701)

DP Barcode: 386043

Reviewers: Christina Wendel, Biologist, EFED, ERB2 Christina Wendel 4/5/11

Kristina Garber, Biologist, EFED, ERB2 4/5/11

Date: April 5, 2011

Purpose: There were concerns with the solubility of the compound (0.5 ppm), and as a result all aquatic studies were further reviewed to check validity, specifically relating to the measurements of treatment concentrations. In addition, the statistical analysis completed in the original review compared the treatment group(s) to the combined (pooled) control. Therefore, the statistics had to be recalculated comparing the treatment group(s) to the negative control alone.

Method: Statistical analyses were completed using TOXSTAT, as NUTHATCH could not be used, since there was only one treatment group and two controls (negative and solvent). T-tests (in TOXSTAT) were used to determine if there were significant differences between the solvent and negative controls. To estimate the EC_{50} and NOAEC, both Dunnett's and Tukey Test of multiple comparisons were used to compare the means of the treatment groups independently (in TOXSTAT).

Results: The study results originally reported for the *Skeletonema costatum* algal toxicity study indicated that the nominal concentration of 11.0 mg/L significantly reduced the cell growth of *S. costatum* over a five-day period, there was a 15.1% cell growth inhibition as compared to the combined (pooled) control (see Table 1). The original reviewers also reported that there were no differences between the two control groups; however they questioned this result due to the large variability in the dataset. The original reviewers also reported that the treatment solution contained particulate matter throughout the majority of the test, but believed that the material "was present at its maximum solubility (0.5 mg/L)." The original reviewers determined that the EC_{50} was greater than 11.0 ppm, and the study was classified as acceptable meeting guideline requirements for Tier I non-target aquatic plant study using *S. costatum*.

The new analysis compared the treatment group to the negative control, as only one test concentration was used; it was represented as a potential limit test. There were no significant differences in the mean standing crop (cells/mL) between the negative and solvent control (see appendix 1); there was a 3.4% growth in the solvent control, relative to the negative control (see Table 2).

Study Classification: The study is now classified as invalid.

Table 1. *S. costatum* reported measurements from the study, and percent inhibition calculation using the solvent control as reported in the original DER for this study.

Nominal Concentration, mg/L	Mean Standing Crop on day 5, cells/mL	Percent Inhibition
Combined Control	240,728	--
11.0	204,337	15.1%

Table 2. *S. costatum* reported measurements from the study, and recalculated percent inhibition calculation using the negative control.

Nominal Concentration (mg/L)	Mean Cell Counts (cells/mL) Day 5	Percent Inhibition Day 5
Negative Control	236,667 (±3.71E+03)	--
Solvent Control	244,790 (±2.56E+04)	-3.43%
11.0	213,337 (±2.72E+03)	10.3%
11.0 (Corrected for blank)	204,337 (±2.72E+03)	13.7%

(± SD) - Standard deviation

[1] A negative percent inhibition indicates stimulation.

Reviewer Comments: This study was originally reviewed by Michael Davy and Daniel Rieder in 1994. The details of the method of this study are provided in the original DER for this study.

The aquatic plant toxicity study using *S. costatum* was originally classified core (*i.e.*, acceptable).

The **aquatic plant toxicity study using *S. costatum* is reclassified as invalid** because of the following:

1) The test substance was not completely in solution, and the electronic particle counter could not distinguish between algae and the other particulates. To correct for this, cell counts of the treatment group were adjusted based on counts obtained in the test substance blank (prepared at concentration of 11.0 mg/L, but with no algae).

2) The actual concentration that the test organism was exposed to is unknown because:
   - The nominal treatment concentration was 11.0 mg/L. The test concentrations were not measured during the study.

- At test initiation and throughout the test the treatment solution appeared cloudy with white particulates.
- The test material was neither centrifuged nor measured.
- It is likely that the concentration that the test organisms were exposed to was at least the solubility limit of DCPA in water (0.5 mg/L; U.S. EPA 1998), but it is not known for certain.

3) The percent inhibition of the mean standing crop (cells/mL) between the solvent control as compared to the negative control was -3.4% (indicating a slight stimulation of growth) (see appendix 1). The percent inhibition of the mean standing crop (cells/mL) between the treatment as compared to the negative control was 13.7%, was not significantly different (see appendix 1). The percent inhibition of the mean standing crop (cells/mL) between of the treatment as compared to the solvent control was 16.5% (see appendix 1).

References:

U.S. EPA. 1998. *Reregistration Eligibility Decision (RED): DCPA.* EPA 738-R-98-005. November 1998. Special Review and Reregistration Division, Office of Pesticide Programs. Washington, D.C. U.S.A.

Appendix 1. Statistical Analysis of *Skeletonema costatum* toxicity data

```
Title:  DCPA Skeletonema Tox
File:          DCPASKEL
  t-Test of Solvent and Blank Controls    Ho: GRP1 Mean = GRP2 Mean
-------------------------------------------------------------------------
  GRP1 (Solvent cntl) Mean = 236666.6667   Calculated t value = -0.5449
  GRP2 (Blank cntl) Mean   = 244790.0000   Degrees of freedom =  4
  Difference in means          = -8123.3333
-------------------------------------------------------------------------
  2-sided t value (0.05, 4) = 2.7764      No significant difference at alpha=0.05
  2-sided t value (0.01, 4) = 4.6041      No significant difference at alpha=0.01
  WARNING: This procedure assumes normality and equal variances!

Title:  DCPA Skeletonema Tox
File:          DCPASKEL          Transform:          NO TRANSFORMATION
                              ANOVA Table
-------------------------------------------------------------------------------
     SOURCE             DF           SS                MS              F
-------------------------------------------------------------------------------
     Between             2     2747689622.5547  1373844811.2773      6.1131
     Within (Error)      6     1348430933.0000   224738488.8333
-------------------------------------------------------------------------------
     Total               8     4096120555.5547
-------------------------------------------------------------------------------
                                                        (p-value = 0.0357)
     Critical F  = 10.9248 (alpha = 0.01, df = 2,6)
                 = 5.1433 (alpha = 0.05, df = 2,6)
     Since  F > Critical F  REJECT  Ho: All equal (alpha = 0.05)
```

```
  Title:  DCPA Skeletonema Tox
  File:          DCPASKEL              Transform:          NO TRANSFORMATION
        Dunnett's Test   -   TABLE 1 OF 2             Ho:Control<Treatment
-------------------------------------------------------------------------------
                           TRANSFORMED     MEAN CALCULATED IN           SIG
GROUP   IDENTIFICATION          MEAN          ORIGINAL UNITS    T STAT  0.05
----- -------------------   ----------   ------------------  ------  ---
    1          neg control   236666.6667       236666.6667
    2         solv control   244790.0000       244790.0000      -0.6637
    3                 11.0   204336.6667       204336.6667       2.6413  *
-------------------------------------------------------------------------------
  Dunnett critical value = 2.3400   (1 Tailed, alpha = 0.05, df = 2,6)

  Title:  DCPA Skeletonema Tox
  File:          DCPASKEL              Transform:          NO TRANSFORMATION
        Dunnett's Test   -   TABLE 2 OF 2             Ho:Control<Treatment  -------
-------------------------------------------------------------------------
                           NUM OF    MIN SIG DIFF     % OF     DIFFERENCE GROUP
IDENTIFICATION      REPS   (IN ORIG. UNITS)  CONTROL  FROM CONTROL
----- -------------------  -------  ----------------  -------  ------------
    1          neg control      3
    2         solv control      3          999.9999        0.4    -8123.3333
    3                 11.0      3          999.9999        0.4    32330.0000
-------------------------------------------------------------------------------
  NOTE: MSD = 999.9999 means actual MSD estimate > 999.

  Title:  DCPA Skeletonema Tox
  File:          DCPASKEL              Transform:          NO TRANSFORMATION
                   Tukey Method of Multiple Comparisons
-------------------------------------------------------------------------------
                                                      GROUP
                          TRANSFORMED  ORIGINAL       0 0 0
  GROUP  IDENTIFICATION      MEAN        MEAN         3 1 2
  -----  --------------  -----------  ---------      - - -
    3              11.0 204336.6667204336.6667      \
    1       neg control 236666.6667236666.6667      . \
    2      solv control 244790.0000244790.0000      * . \
-------------------------------------------------------------------------------
* = significant difference (alpha = 0.05)       . = no significant difference
  Tukey critical value = 4.3390 (df = 3,6)              s = 224738488.833
```

DATA EVALUATION RECORD

1. **CHEMICAL:** Chlorthal Dimethyl.
Shaughnessey No. 078701.

2. **TEST MATERIAL:** DCPA technical (dimethyl tetrachloroterephthalate); CAS No. 1861-32-1; Lot No. 10148/T-170-2; 98.4% active ingredient; a tan powder.

3. **STUDY TYPE:** 122-2. Growth and Reproduction of Aquatic Plants - Tier 1. Species Tested: *Skeletonema costatum.*

4. **CITATION:** Hughes, J.S. and P.H. Balcom. 1993. The Toxicity of DCPA Technical to *Skeletonema costatum.* Laboratory Project ID No. B038-033-3. Conducted by Malcolm Pirnie, Inc., Tarrytown, NY. Submitted by ISK Biotech Corporation, Mentor, OH. EPA MRID No. 428361-03.

5. **REVIEWED BY:**

Michael W. Davy **Signature:** Michael Davy
Agronomist
Ecological Effects Branch **Date:** 3/25/94
Environmental Fate and Effects Division

6. **APPROVED BY:**

Daniel Rieder **Signature:** Daniel Rieder
Section Head
Ecological Effects Branch **Date:** 5-12-94
Environmental Fate and Effects Division

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a Tier 1 non-target aquatic plant study using *Skeletonema costatum.* Based on the nominal concentrations, the EC_{50}> 11.0 ppm during the 5-day test period.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. MATERIALS AND METHODS:

A. Test Species: The diatom used in the test, *Skeletonema costatum*, came from laboratory stock cultures originally obtained from the EPA Environmental Research Laboratory in Gulf Breeze, FL. Stock cultures were maintained in synthetic marine algal assay nutrient medium (MAA) under 4306 lux illumination at a temperature of 20 ±2°C. The photoperiod was 14 hours of light per day. The cultures were manually shaken each working day. Transfers were made regularly to provide logarithmically-growing cultures. The culture used as inoculum in this test had been transferred to fresh medium seven days before test initiation.

B. Test System: All glassware was cleaned and autoclaved before use. Test vessels used were 250-ml Erlenmeyer flasks fitted with foam stoppers which permitted gas exchange. The test medium was the same as that used for culturing with the pH adjusted to 8.1 ±0.1. The medium was filter sterilized (0.22 µm) prior to inoculation.

The test vessels were kept in an incubator under environmental conditions like those employed in culturing with 14 hours of cool-white fluorescent illumination per day.

A 22 mg active ingredient (ai)/ml stock solution was prepared by dissolving 559.1 mg of the test material in N,N-dimethylformamide (DMF) and diluting to a final volume of 25 ml. The test solution was prepared by adding 0.125 ml of the stock to 0.25 l of nutrient medium. A second set of treatment solutions (test material but no algal inoculum) was also prepared to serve as the blank for particle counting.

C. Dosage: Five-day growth and reproduction test. One nominal concentration of 11 mg ai/l was selected for the test. A solvent control (0.5 ml DMF/l of nutrient solution) and a medium control were also prepared. The maximum labeled application rate for DCPA was reported to be 15 lb ai/acre. This is equivalent to 11.0 mg ai/l if applied to a 15-cm water column.

D. Test Design: Fifty ml of the appropriate test or control solution were placed into each of three replicate flasks for each treatment and control.

The cellular density of an *S. costatum* culture was determined. An inoculum of cells calculated to provide 10,000 cells/ml was aseptically introduced into each flask. The inoculum volume was 0.977 ml per flask. The flasks were manually shaken and randomly repositioned each working day to minimize spatial differences in the incubator. Cell counts were performed using an electronic particle counter on test days 3, 4, and 5. Three counts were made per replicate.

The pH was measured at test initiation and termination. Temperature was monitored manually daily and continuously with a recording device. Analytical measuremets of the test material in the treatment solutions were not performed.

E. **Statistics:** Percentage inhibition was determined by comparison of the terminal treatment cell number to that of the pooled control. If the treatment resulted in inhibition of greater than or equal to 50%, then Tier 2 testing is indicated.

12. **REPORTED RESULTS:** Throughout the test (with the exception of day 5), particulates were noted in the treatment solutions. The treatment concentration (11 mg ai/l) was 22 times greater than the reported maximum water solubility of DCPA (0.5 mg ai/l).

Cell counts and percentage inhibition after five days are given in Tables 3 and 4 (attached). Percentage cell growth inhibition was 15.1% in comparison to the pooled control.

The pH was 7.93 in the test solutions at study initiation. The pH values on day 5 ranged from 8.72 to 8.82.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The authors concluded that Tier 2 testing was not required due to less than 50% inhibition observed at the tested concentration of 11 mg ai/l.

Good Laboratory Practice and Quality Assurance statements were included in the report indicating compliance with EPA Good Laboratory Practice Standards, 40 CFR Part 160.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines, except for the following deviations:

Cell growth measurements were not taken daily. Measurements were made on days 3, 4, and 5 only.

The results of the daily or continuous temperature measurements were not reported.

The photoperiod (14 hours of light per day) was less than recommended (16 hours of light per day).

B. **Statistical Analysis:** The reviewer used a t-test to determine if a significant difference in cell number existed between the two controls and between the pooled control and treatment. The results of the analysis indicated that there was no significant difference between the two control groups. However, this comparison was in question due to the large differences between the variances about the mean. This was also the case when the pooled control data and the treatment data were compared. Therefore, the control data was compared to the treatment data, and a significant reduction was detected. Therefore, DCPA technical at a nominal concentration of 11 mg ai/l significantly reduced the cell growth of *S. costatum* over a five day period (see attached printouts).

C. **Discussion/Results:** The treatment solution contained particulate matter throughout the majority of the test. The reviewer believes that the material was present at its maximum solubility (0.5 mg ai/l).

This study is scientifically sound and meets the guideline requirements for a Tier 1 non-target aquatic plant study. Based on the nominal concentrations, the EC_{50}> 11.0 ppm during the 14-day test period.

D. **Adequacy of the Study:**

(1) Classification: Core

(2) Rationale: N/A

(3) Repairability: N/A

15. **COMPLETION OF ONE-LINER:** Yes

DATA EVALUATION RECORD

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Shaughnessey No. 078701.

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5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Agronomist
KBN Engineering and
Applied Sciences, Inc.

Signature: Mark A. Mossler
Date: 9/27/93

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 9/27/93

~~Henry T. Craven~~, M.S.
Supervisor, EEB/EFED
USEPA

Signature: Henry Craven
Date: 3 24 94

7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for a Tier 1 non-target aquatic plant study. The actual concentration of DCPA technical in solution was not determined. Based on the maximum water solubility of the test material (0.5 mg ai/l), the cellular growth of *S. costatum* was significantly reduced (15.1%) during the 5-day test period.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

DCPA Technical: *Skeletonema costatum* Toxicity Test

Table 3. Cell counts[1] (cells/mL) during test

Nominal Concentration, mg/L		Day 3 3-8-93	Day 4 3-9-93	Day 5 3-10-93
No-Treatment Control	A	143,410	205,780	237,340
	B	144,960	202,310	239,990
	C	135,440	195,360	232,670
	Mean	141,270	201,150	236,667
	SD[2]	5.11E+03	5.31E+03	3.71E+03
	Var[3]	2.61E+07	2.82E+07	1.37E+07
Solvent Control	A	159,210	234,170	270,310
	B	146,310	209,140	244,860
	C	135,100	192,850	219,200
	Mean	146,873	212,053	244,790
	SD	1.21E+04	2.08E+04	2.56E+04
	Var	1.46E+08	4.33E+08	6.53E+08
11.0	A	90,890	149,120	210,310
	B	85,660	148,560	215,590
	C	94,570	153,490	214,110
	Mean	90,373	150,390	213,337
	SD	4.48E+03	2.70E+03	2.72E+03
	Var	2.00E+07	7.29E+06	7.42E+06
Blank		22,000	12,000	9,000
11.0 (Corrected for blank)	A	68,890	137,120	201,310
	B	63,660	136,560	206,590
	C	72,570	141,490	205,110
	Mean	68,373	138,390	204,337
	SD	4.48E+03	2.70E+03	2.72E+03
	Var	2.00E+07	7.29E+06	7.42E+06

[1] Each value represents the mean of three sample counts

[2] SD = standard deviation

[3] Var = variance

DCPA Technical: *Skeletonema costatum* Toxicity Test

Table 4. Percent inhibition, relative to combined control, based upon mean standing crop, cells/mL, on day 5

Nominal Concentration, mg/L	Mean Standing Crop on day 5, cells/mL	Percent Inhibition
Combined Control	240,728	---
11.0	204,337	15.1

```
                    º STUDENT'S T-TEST (two-tailed) º
                    ÈÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍ¼

        Enter the name of the DATAFILE you wish to analyze:  skl
      (Press RETURN if you wish to skip directly to T evaluation)

   What are the SAMPLE NUMBERS of the 2 variables you want to compare?
                 1 `c'                          2 `sc'
    Means =        236666.7                       244790
Variances =        1.373564E+07                   6.530616E+08

         Are these INDEPENDENT or PAIRED samples? (I or P)   i

The T-TEST may not be appropriate because these variances
                 are so different (F = 47.54506   p = 2.059943E-02 ).

           T =  .5448698                              df =  4
                                 p =  .6147984
       The MEANS of these 2 samples are NOT significantly different.

The confidence limits on the DIFFERENCE between the means of these samples
can be calculated as:
                          8123.328 +/- T(4) *  14908.75

              Do you want another T-TEST using this datafile?
```

```
                    º STUDENT'S T-TEST (two-tailed) º
                    ÈÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍ¼

        Enter the name of the DATAFILE you wish to analyze:  skl
      (Press RETURN if you wish to skip directly to T evaluation)

   What are the SAMPLE NUMBERS of the 2 variables you want to compare?
                1 `pooled'                    2 `trt'
    Means =        240728.3                     204336.7
Variances =        2.865155E+08                 7418133

         Are these INDEPENDENT or PAIRED samples? (I or P)   i

The T-TEST may not be appropriate because these variances
                are so different (F = 38.62367   p = 2.542901E-02 ).

           T =  3.578966                          df =  7
                                p =  8.988501E-03
       The MEANS of these 2 samples are significantly different.

The confidence limits on the DIFFERENCE between the means of these samples
can be calculated as:
                          36391.66 +/- T(7) *  10168.21

              Do you want another T-TEST using this datafile?
```

```
                    É͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸»
                    º STUDENT'S T-TEST (two-tailed) º
                    È͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸͸¼

       Enter the name of the DATAFILE you wish to analyze:  skl
     (Press RETURN if you wish to skip directly to T evaluation)

   What are the SAMPLE NUMBERS of the 2 variables you want to compare?
                1 `control'                    2 `trt'
    Means =        236666.7                      204336.7
Variances =        1.373564E+07                  7418133

        Are these INDEPENDENT or PAIRED samples? (I or P)    i

          T =  12.17097                                df =  4
                                p =  2.614856E-04
       The MEANS of these 2 samples are significantly different.

The confidence limits on the DIFFERENCE between the means of these samples
can be calculated as:
                          32330 +/- T(4) *  2656.321


                Do you want another T-TEST using this datafile?
```